11:00

## 790-3 Induction of Programmed Cell Death in Human Vascular Smooth Muscle Cells Following Exposure to β Irradiation

A. Rivard, M. Bouchard, R. Martel, A. Fleser, P. Kermani, G. Leclerc. *Notre-Dame Hospital, Montréal, Québec, Canada*

Relevant biological mechanisms by which endovascular irradiation may prevent neointimal hyperplasia following angioplasty remain unclear. Radiation-induced programmed cell death may play an important role in this phenomenon. Accordingly, the purpose of this study was to verify if $^{32}P$ irradiation induces apoptosis in vascular smooth muscle cells (VSMC). Human VSMCs were irradiated with sealed sources of $^{32}P$ with activity levels of 0.2, 0.5, 1, 2, and 5 μCi, for continuous periods of 6, 12, and 24 hours. Apoptosis was evaluated by labelling the cell's DNA with $^{14}C$-thymidine and then comparing the level of incorporated $^{14}C$ in fragmented and intact DNA. The irradiation effect on cellular proliferation was also assessed under the same conditions using the $^{3}H$-thymidine assay. No significant apoptosis was detected for irradiation periods of 6 and 12 hours. Similarly, no inhibition of cellular proliferation was detected for the same exposure time to $^{32}P$. After 24 hours of irradiation, however, apoptosis was detected for the 2 and 5 μCi activity levels, with respective values of 23.5 ± 3.8% (P = 0.008 vs control) and 26.4 ± 5% (P = 0.002 vs control). Inhibition of VSMC proliferation was significative after the 24 hour exposure period with inhibition values of, respectively, 31.9 ± 4.9% (P = 0.001 vs control) and 62.5 ± 6.3% (P = 0.0002 vs control) for the 2 and 5 μCi activities. No significant apoptosis was detected for the lower activity of $^{32}P$ source. *Conclusions:* 1. Exposure of human VSMCs to β radiation originating from a $^{32}P$ source results in a significant reduction in their proliferation index. 2. Concomitant with this proliferation inhibition, β irradiation induces apoptosis in VSMCs. 3. The occurrence of apoptosis and inhibition of proliferation in VSMCs by β irradiation, appears to be dependent on a threshold of source activity and exposure time.

11:15

## 790-4 Atherectomy Specimens Obtained From Patients With Restenotic Lesions Reveal Higher Monocyte Chemoattractant Protein-1 Levels Than Those With De novo Lesions

M.M. Rozek, B. Chandrasekar, R.S. Kiesz, G.L. Freeman. *University of Texas, Health Science Center at San Antonio, TX, USA*

This study sought to study the presence of monocyte chemoattractant protein 1 (MCP-1) in the human atherectomy specimens derived from both de novo and restenotic lesions.

*Methods:* A total of 24 specimens (12 de novo and 12 restenotic) were used in this study. Frozen sections (5 μ) were used for immunostaining (IHC), using an immunoenzymatic staining kit (DAKO). As a primary antibody rabbit anti baboon MCP-1 polyclonal antibody was used in 1:750 dilution. Counterstaining was performed with Mayer's hematoxylin. IHC staining was evaluated by light microscopy, grading on a semiquantitative scale from 0 to 4 in a blinded manner, corresponding to the estimated fraction of positive staining cells and the estimated average staining intensity of positive cells, respectively. Staining was assessed and recorded according to a proportion and intensity scoring system developed for IHC staining of tumors (Allred et al, 1993).

*Results:* staining for MCP-1 was present in 25% of the specimens obtained from de novo lesion, staining index was between 1 and 2. On the other hand, MCP-1 stain in restenotic lesion was present in all specimens, with the intensity of stain 2–3. Cells producing MCP-1 were identified by monoclonal antibodies as smooth muscle cells and monocytes/macrophages.

*Conclusions:* MCP-1 is produced by smooth muscle cells and monocyte/macrophages in coronary atheromas. Since a significant difference was shown between de novo and restenotic lesions, the pivotal role of monocyte/macrophages in restenosis processes is suggested.

11:30

## 790-5 Adenoviral Gene Transfer of Human Constitutive Endothelial Nitric Oxide Synthase to Injured Coronary Arteries

O.H. Varenne, S. Pislaru[1], H. Gillijns, R.D. Gerard, F. Van de Werf[1], D. Collen, S.P. Janssens[1]. *Center for Transgene Technology and Gene Therapy, Katholieke University of Leuven, Leuven, Belgium, [1] Department of Cardiology Katholieke University of Leuven, Leuven, Belgium*

Coronary gene transfer to prevent restenosis has been hampered by inefficient local delivery systems. We assessed the ability of the Infiltrator catheter to deliver adenovirus to the porcine LAD after overstretch injury (20 mm balloon, 3 inflations, 10 atm). Virus encoding either β-galactosidase (AdCMVβgal) or nitric oxide synthase (AdCMVceNOS) was injected (0.3 ml of 5 × $10^9$ pfu/ml) over 10–15 sec. Histological staining for β-galactosidase and ceNOS showed homogeneous transgene expression in medial smooth muscle cells (SMC) and in adventitial cells adjacent to the media. A maximum of 41 ± 10% of medial SMCs and 23 ± 3% of adventitial cells expressed the transgene. Thus, the Infiltrator catheter enables highly efficient intramural adenovirus-mediated gene transfer. No staining was observed in the distal LAD, unrelated coronary arteries, or arteries infected with control adenovirus lacking a transgene (AdRR5). AdCMVceNOS infection markedly reduced platelet adhesion at the site of injury as studied by anti-platelet glycoprotein Ib immunostaining. Neointima formation was assessed at 28 days by computer-assisted planimetry on 6 μm sections. Balloon injury was similar in the two groups (balloon:artery ratio of 1.60 ± 0.08 vs 1.65 ± 0.09, p = NS). Initial studies (being extended) suggest that the neointimal area was reduced in AdCMVceNOS-treated vessels (0.75 ± 0.22 $mm^2$ vs 1.24 ± 0.36 $mm^2$ in AdRR5, $p = 0.058$, $n = 8$). Overexpression of recombinant ceNOS in balloon-injured coronary arteries may be a promising therapeutic strategy for restenosis.

11:45

## 790-6 Urokinase Plasminogen Activator Expression After Balloon Injury is Associated with Adventitial Cell Migration and Angiogenesis of the Vasa Vasorum

K. Pels, M. Labinaz, M.R. Garvin, C. Hoffert, E.R. O'Brien. *Univ. Ottawa Heart Inst., Ottawa, Canada*

In a porcine model of coronary artery angioplasty, we have observed angiogenesis of the vasa vasorum and adventitial myofibroblast proliferation and apparent inward migration. Before a cell can migrate local tissue barriers must be broken down by proteases, such as urokinase plasminogen activator (uPA). *Purpose:* Determination of the temporal relation between uPA expression and adventitial cell migration. uPA and plasminogen activator inhibitor (PAI-1) expression was studied by *in situ* hybridization and immunocytochemistry on days 3, 7, 14 and 28 after single and double injury of porcine coronary arteries. Incorporation of BrDU was used to assess cell proliferation. *Results:* Maximal levels of proliferating cells (e.g., ≤ 4%) were found in either the combined intima + media, or adventitia 3 days after single or double injury. Adventitial microvessel number and area increased dramatically 3 days after injury – only to later undergo resorption. Despite an absence of proliferation, intimal and medial cell number peaked on day 28 after second injury – probably due to inward migration of adventitial cells. PAI-1 mRNA was expressed in both normal and diseased arteries. uPA mRNA and protein expression showed a discrete interval of upregulation on days 3 and 7 after balloon injury – particularly in adventitial myofibroblasts and endothelial cells, and then returned to near baseline levels.

*Conclusions:* After angioplasty, uPA over-expression coincides with adventitial angiogenesis and the apparent inward migration of myofibroblasts. Antagonism of the uPA receptor may provide insight into the role of uPA in vascular cell migration and lesion formation.

# 791 Unstable Angina: Pathophysiology II

Wednesday, March 19, 1997, 2:00 p.m.–3:30 p.m.
Anaheim Convention Center, Room C2

2:00

## 791-1 Circadian Variation of Nitric Oxide Production in Normal Subjects

M. Karita, H. Sato, Y. Koretsune, H. Yokoyama, Y. Ohnishi, T. Kurotobi, M. Kitakaze, K. Node, M. Hori. *The First Dept. of Med., Osaka Univ. Osaka, Japan*

The time of the onset of acute myocardial infarction has circadian variation, i.e., the incidence of acute myocardial infarction is highest during the period from 6 a.m. to noon. Nitric oxide (NO) plays an important role in preventing against thrombosis by regulating platelet-vessel wall interaction. Therefore, if NO production is decreased in the morning, it may be a pivotal role for the high incidence of acute myocardial infarction in this period. The aim of this study was to determine whether a circadian variation is detected in the plasma NO levels. We studied 10 healthy male volunteers, aged 23–40. Blood was sampled from the peripheral vein at 4-hour intervals for 24 hours. We measured the plasma concentration of nitrate plus nitrite ($NO^{2-}$ + $NO^{3-}$), the end-product of NO, by Griess method. $NO^{2-}$ + $NO^{3-}$ concentration was decreased in the morning and was lowest at 12:00. The lowest concentration was about a half as compared with a peak at 16:00 (Fig.).